

526,576

10/526576
Rec'd PCT/PTO 04 MAR 2005

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau(43) International Publication Date
18 March 2004 (18.03.2004)

PCT

(10) International Publication Number
WO 2004/022678 A1(51) International Patent Classification⁷: C11B 1/10,
1/06, A23D 9/00, C12P 7/64, A23L 1/30, A23K 1/16,
A23C 11/04(21) International Application Number:
PCT/EP2003/008745

(22) International Filing Date: 7 August 2003 (07.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02019908.9 4 September 2002 (04.09.2002) EP(71) Applicant (for all designated States except US): NESTEC
S.A. [CH/CH]; Avenue Nestlé 55, CH-1800 Vevey (CH).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BERTHOLET,
Raymond [CH/CH]; Ch. Vers-Chez-Cochard 11, CH-1807
Blonay (CH). WANG, Junkuan [CH/CH]; Chemin des
Abbeses 2B, CH-1027 Lonay (CH). WATZKE, Herib-
ert, Johann (J.) [AT/CH]; Ch. de la Fontannetaz 21, CH-
1009 Pully (CH). GERMAN, John, Bruce [CA/CH];
Résidence la Bergerie, CH-1072 Forel/Lavaux (CH).(74) Agent: ARCHAMBAULT, Jean; Avenue Nestlé 55,
CH-1800 Vevey (CH).(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE,
SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, YU, ZA, ZM, ZW.(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)
- of inventorship (Rule 4.17(iv)) for US only

Published:

- with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PROCESS FOR PREPARING AN OIL CONTAINING ONE OR MORE LONG-CHAIN POLYUNSATURATED FATTY ACIDS DERIVED FROM BIOMASS; FOODSTUFF OR NUTRITIONAL, COSMETIC OR PHARMACEUTICAL COMPOSITION CONTAINING IT

(57) Abstract: A stable oil containing LC-PUFAs in the form of triacylglycerols, in particular arachidonic acid (ARA), dihomogammalinolenic acid (DHGLA), docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA), may be prepared by direct pressing of one or more biomasses obtained from the culture of a microorganism, especially of a fungus or of a microalga containing the acids ARA, DHGLA, DHA or EPA leading to a first press oil and by bringing a carrier oil entering into the composition of a foodstuff, a cosmetic or pharmaceutical product, into contact with the biomass cake, followed by pressing leading to a second press oil, and then by combining the pressed oils and refining the mixture under controlled conditions.



WO 2004/022678 A1

Process for preparing an oil containing one or more long-chain polyunsaturated fatty acids derived from biomass; foodstuff or nutritional, cosmetic or pharmaceutical composition containing it

5

Introduction

The present invention relates to the field of the preparation of an oil serving as an ingredient which is a source of long-chain polyunsaturated essential fatty acids (LC-PUFAs) in a foodstuff, in a nutritional supplement, in a cosmetic or pharmaceutical composition.

15 State of the art

An oil containing LC-PUFAs such as for example arachidonic acid (ARA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) or dihomogammalinolenic acid (DHGLA) may be obtained from a biomass fermentation broth. To obtain the oil from the biomass, methods of extraction with organic solvent, for example hexane, or with supercritical fluid, have been used. Generally, the oil has been extracted from biomass by percolation of the dried biomass with hexane.

Such a process of extraction with organic solvent(s) is described, for example, in WO9737032, in WO 9743362 or in the publication Journal of Dispersion Science and Technology, 10, 561-579, 1989 "Biotechnological processes for the production of PUFAs".

This technique has various disadvantages:

- During the stages of extraction with hot solvent or of distillation of the solvent, the LC-PUFAs may undergo degradation in contact with oxygen.
- The complete removal of the solvent contained in the oil or in the residual biomass requires a

heat treatment at high temperature.

- Moreover, the solvent, such as hexane, is capable of dissolving nontriacylglycerol constituents of the biomass which in fact
5 constitute impurities.

The crude oil obtained after evaporation of the solvent should further undergo several refining stages comprising degumming, neutralization with alkali,
10 decolorization, dewaxing and deodorization with the aim of at least partially removing the impurities. This means that the highly unsaturated oil is exposed to conditions stimulating physicochemical reactions which affect its quality. For example, the decolorization
15 agents create a system of conjugated double bonds and form degradation products by chemical reaction with the oxidized glycerides.

A process for extracting biomass with no solvent is also known, for example from EP-A-1178118. According to
20 this process, the use of solvent is avoided by preparing an aqueous suspension of the biomass and by separating an oily phase, containing the desired oil, from the aqueous phase, by centrifugation. The aqueous
25 phase contains cell wall debris and a quantity of water-soluble material obtained from the biomass. A disadvantage of this process is that the crude oil obtained is contaminated with quantities of impurities, for example polar lipids, residues of proteins due to
30 the presence of water. Such a crude oil should then be refined by conventional methods for refining vegetable oils.

The aim of the present invention is to avoid the
35 disadvantages of the prior art, by providing a process for preparing a stable oil containing one or more polyunsaturated fatty acids derived from biomass in the form of triacylglycerols in the purified state with a high yield and in which the oil undergoes minimum

degradation.

Summary of the invention

5 The present invention relates to a process for preparing a stable oil containing LC-PUFAs in the form of triacylglycerols, in particular arachidonic acid (ARA), dihomogammalinolenic acid (DHGLA), docosahexaenoic acid (DHA) or eicosapentaenoic acid
10 (EPA).

The process is characterized in that one or more biomasses obtained from the culture of a microorganism, especially of a fungus or of a microalga containing the
15 acids ARA, DHGLA, DHA or EPA, are pressed in the dry state so as to obtain a first press oil and a cake, and in that the oil thus obtained is treated with an adsorbent and in that it is subjected to deodorization under controlled conditions.

20 The oil thus obtained does not require chemical refining. Treatment with an adsorbent, for example a silicate, and deodorization under gentle conditions are sufficient to obtain a purified and stable oil which
25 can be used as a nutritional supplement.

According to one preferred embodiment of the process,
- the press cake is placed in contact with a carrier oil entering into the composition of a foodstuff
30 and the mixture of the carrier oil and the cake is pressed in order to obtain a second press oil, and
- the pressed oils are mixed in variable proportions so as to obtain an LC-PUFA concentration which is appropriate for the specific application. The
35 mixture is then subjected, where appropriate, to physical refining and to deodorization.

Preferably, the oil does not contain more than 10% by weight of LC-PUFAs. As a result, the oil is a lot less

sensitive to oxidation during its production, which is not the case for the oils containing LC-PUFAs of the prior art.

- 5 According to a main aspect of the invention, it is a crucial qualitative advantage to have available a novel oil containing LC-PUFAs in the form of triacylglycerols.
- 10 According to another aspect, the invention relates to a foodstuff, a cosmetic or pharmaceutical product, a nutritional supplement or an animal feed containing the preceding oil.
- 15 According to yet another aspect, the invention relates to an animal feed, in particular for pets, containing the biomass residue derived from the process.

Detailed description of the invention

- 20 The preparation of the oil is carried out by direct pressing of a biomass containing LC-PUFAs and production of a first press oil. In order to increase the LC-PUFA yield, the biomass cake obtained from the
- 25 direct pressing is brought into contact with a carrier oil and the mixture is subjected to pressing in order to obtain a second press oil. The two pressed oils are then mixed in variable proportions so as to obtain an LC-PUFA concentration which is appropriate for the
- 30 specific application. The mixture is then subjected, where appropriate, to physical refining and the desired oil is thus obtained.

- Physical refining, in the context of the invention, is
- 35 aimed at a reduction in the phospholipids and the free fatty acids and is understood to mean a degumming treatment without the use of acid and without neutralization.

By way of example, it was observed that in the case of a biomass containing ARA, the first press oil was already free of most free fatty acids and phospholipids and, consequently, depending on the desired degree of purity, did not require degumming treatment.

On the other hand, in the case of a biomass containing DHA for example, such a degumming treatment was necessary in order to reduce, in particular, the phospholipids so as to obtain the desired degree of purity.

The oil obtained is suitable for application in foodstuffs; in particular infant formulas or for use as a nutritional supplement. It may also be used in cosmetic or pharmaceutical products. Furthermore, the biomass residue obtained is also a product of the process which may be upgraded directly without subsequent treatment, for example as animal feed, in particular for pets.

The preparation of such an oil may take place by simply pressing the dried biomass. Preferably, the carrier oil is mixed with the residue of the first pressing of the biomass and the oil is subsequently separated from the nonlipid solids by pressing.

In order to increase the yield of LC-PUFA obtained, it is preferable to reduce the sizes of the particles of dry biomass in order to break the walls of the cells of microorganisms and to thereby release the oil. It is possible, in the preferred embodiment, to reduce the sizes of the particles of the press cake in order to increase the surface area of contact between the carrier oil and the biomass residue. This may be appropriately carried out using various methods, for example:

- the biomass cake may be ground in the presence of the carrier oil;

- the biomass cake may be laminated before mixing it with the carrier oil;
- the biomass cake may be treated at high pressure in the presence of the carrier oil, and then the oil obtained may be separated from the biomass cake by pressing and final filtration;
- the biomass or cake may be treated with enzymes capable of degrading the walls of the cells.

10 Because the carrier is an oil, the oil obtained after contact with the biomass cake has a minimum content of phospholipids, free fatty acids, pigments, polymers and other substances obtained or derived from the biomass which are not triacylglycerols. This means that the process according to the invention constitutes a selective method for preparing a stable purified oil containing LC-PUFAs. It is not necessary to purify the unsaturated oil containing the LC-PUFAs by the aggressive and cumbersome methods used prior to the invention such as the stages of degumming, neutralization, dewaxing and decolorization.

According to the invention, the pressed oils are subjected, where appropriate, to a refining step using a processing agent, for example a silicate. The treatment with the processing agent may be carried out during contact with the carrier oil or after production of the pressed oil, for example during filtration. Finally, the oils are subjected to a stage of deodorization, for example by steam distillation or molecular distillation at a relatively low temperature. The result is that the oil obtained contains a particularly small quantity of trans fatty acids.

35 The process does not use organic solvent and, since the operation is carried out under a nitrogen layer and in the presence of tocopherols or tocotrienols which are naturally present in or which are added to the carrier oil, the LC-PUFAs are protected from oxidative

degradation during the whole process.

In addition to the quality of the oil obtained, another advantage of the process consists in the fact that the biomass residue is not contaminated with an organic solvent and may thus be directly upgraded, without subsequent treatment, for example in animal feed, in particular for pets.

The detailed description of the process which follows is targeted at the preparation of an oil containing ARA, an oil containing DHA, and an oil containing ARA and DHA, taken by way of nonlimiting examples. The working conditions for transferring other LC-PUFAs to a carrier oil from appropriate biomasses, for example for DHGLA or EPA, are very similar.

In the preferred implementation of the process, the oil is obtained by combining the biomass dry-pressing oil and the oil obtained by mixing the carrier oil with the dry biomass pressing residue and separating the oil from the solid components by pressing. To increase the level of incorporation of LC-PUFA, it is desirable to break the microbial cells by high-pressure treatments, by enzymatic processes, or to reduce the sizes of the dry particles of biomass by grinding or laminating.

The grinding step used may be one of many techniques known in the prior art. For example, the biomass may be laminated, preferably at low temperature, and then it may be mixed with the carrier oil. As a variant, the biomass may be ground in the presence of the carrier oil. In order to minimize as much as possible damage to the LC-PUFAs, the grinding conditions should be gentle. In this regard, grinding the biomass in the presence of the carrier oil and under an inert atmosphere, for example under a nitrogen stream, is preferred.

Next, the oil containing the LC-PUFAs is separated from

the biomass cake by filtration or pressing, preferably at high pressure, and then a final filtration is carried out so as to remove the fine particles of biomass residue.

5

It was observed that the level of incorporation of the LC-PUFA increased when the size of the biomass particles decreased; it was > 90% when for example 90% of the particles had a size < 250 μm .

10

By way of example, it is possible to use a ball mill or a colloidal mill. The parameters to be considered are the duration of grinding, the size of the biomass particles, the grinding temperature, the ratio between the quantities of biomass and of carrier oil.

15

The duration of grinding has an influence on the size of the particles and the latter is also influenced by the grinding temperature. Consequently, in practice, it is preferable to indicate the size of the particles as a crucial parameter of the grinding stage. Thus, it is desirable that 90% of the particles have a size < 500 μm , preferably that 90% of the particles have a size < 300 μm and more preferably still that 90% of the particles have a size < 200 μm .

20

25

The grinding temperature is chosen at a value greater than the melting point of the carrier oil, and is preferably from 20 to 80°C.

30

The weight ratio chosen between the biomass and the carrier oil determines the content of LC-PUFA of the final oil. Thus, for example, 30 parts of biomass are chosen per 70 parts of carrier oil in order to obtain at least 3.5% of LC-PUFA in the converted oil.

35

The oil used as carrier may be any oil or mixture of oils which can be consumed as human food. An oil or a mixture entering into the composition of the product

which it is desired to enrich with PUFA is preferably used. There may be mentioned in particular for an infant formula high oleic acid sunflower oil (HOSFO), sunflower oil (SFO), soya bean oil, palm olein and a
5 medium-chain triacylglycerol (MCT, containing essentially triacylglycerols of saturated C₈-C₁₀ fatty acids).

The next stage of the process consists in separating
10 the spent biomass residue by any customary method such as, for example, pressing, filtration or centrifugation. To this end, a press operating at high pressure is preferably used.

15 The oil obtained should be made free of fine insoluble particles by fine filtration. This operation may be carried out, where appropriate, by exposing the oil to a mineral adsorbent as a filter aid, for example dicalite.

20 Finally, the filtered oil is deodorized in order to remove the volatile substances. This may be carried out by any known method provided that moderate conditions are used in order to be gentle on the LC-PUFAs. There
25 may be mentioned, for example, steam distillation, preferably under vacuum, or molecular distillation.

The oil obtained may be used in food compositions for human consumption as it is or in the form of a mixture
30 with other oils such as, for example, a fish oil or such as, for example, salad oils or alternatively in the form of an emulsion in salad dressings or mayonnaises. It may be a constituent of a dietetic milk for teenagers or adults, an infant formula for
35 premature babies, full-term unweaned babies or a follow-on milk for small children.

It may be incorporated into a nutritive or supplemental composition for oral consumption.

It may be incorporated into a pharmaceutical composition for oral, enteral or parenteral ingestion, or for topical, dermatological or ophthalmological application.

It may constitute an ingredient for a cosmetic, topical or oral composition.

Finally, it may constitute an ingredient for a pet food, for example a dry or moist food or a milk.

The biomass residue, after separation of the oil, may be advantageously used in animal feed, particularly for pets.

Examples

The examples below illustrate the invention. Therein, the parts and percentages are by weight, unless otherwise stated.

To determine the % of free fatty acids (FFA), the IUPAC 2.201 method is used,
for the ARA content, the IUPAC 2.304 method is used,
and
for the Phosphorus (P) content, the NI C12-1976-SSOG method is used.

Example 1: Pressing of a biomass containing ARA with a hydraulic press

Equipment:

Carver RC 106 press

Materials used:

Biomass containing 34.7% of oil containing 39.9% of arachidonic acid (ARA)

Procedure:

111 g of the biomass are introduced into a carton cartridge. The cartridge is introduced into the cylinder of the press and the whole is heated in an oven at 70°C for 30 min.

The pressing is then carried out by gradually increasing the pressure up to a final pressure of 700 bar. 23.2 g of clear pressed oil are thus recovered, which corresponds to 60.2% of oil present in the biomass.

The ARA, free fatty acid (FFA) and Phosphorus (P) content of the pressed oil is then determined and it is compared with an oil obtained after hexane extraction of the same biomass. The results are summarized in Table 1 below.

Table 1

	Oil from direct pressing	Oil extracted with hexane
% ARA	39.9	39.9
% FFA	0.1	0.6
mg/kg P	17	508

The oil obtained after direct pressing has an ARA content similar to the oil extracted with hexane but contains less impurities (FFA, P). The pressed oil does not require chemical refining unlike the oil conventionally extracted with hexane. A treatment with an adsorbent (processing agent) followed by deodorization make it possible to obtain a stable oil having a neutral odour.

Example 2: Pressing of a biomass containing DHA with a screw pressEquipment:

Komet screw press

Seitz filter press

Materials used:

Biomass containing 50.5% of oil containing 39% of docosaheptaenoic acid (DHA)

5 Procedure:

150 kg of biomass were pressed in the Komet press under the following conditions:

slit: 8 mm, throughput: 3.75 kg/h

10 A cloudy oil is collected which is filtered using a 20 micron paper filter. 46 kg of a clear oil are thus obtained, which corresponds to 60.7% of oil present in the biomass.

15 The DHA, free fatty acids (FFA) and Phosphorus (P) content of the pressed oil is determined and it is compared with an oil obtained after extracting the same biomass with hexane. The results are summarized in Table 2 below.

20 Table 2

	Oil from direct pressing	Oil extracted with hexane
% DHA	39	39
% FFA	0.7	1.6
mg/kg P	200	630

25 The oil obtained after direct pressing has a DHA content similar to the oil extracted with hexane but contains less impurities (FFA, P). The losses during refining are lower. Treatment with an adsorbent (processing agent) followed by deodorization make it possible to obtain a stable oil having a neutral odour.

30 Example 3: Incorporation of the residual DHA of the pressed biomass into palm olein.

Equipment:

Fryma MZ 80 colloid mill

De Smet minipilot filtration unit with 1.4 l chamber

Seitz 2 l filter press

5 Laboratory deodorizer

Materials used:

Pressed DHA biomass of Example 2 containing 28.2% of oil containing 39% of decosaheanoic acid (DHA)

10 Palm olein

Trisyl® (processing agent)

Procedure:

1.4 kg of the pressed biomass as obtained in Example 2
15 and 2 kg of palm olein are introduced into the container of the mill. The milling is carried out by direct passage through the mill with a slit of 10 microns at a temperature of 40-50°C and the mixture is recovered. 2.6 kg of this mixture are introduced
20 into the filtration unit and filtration is carried out at 50°C, and then the cake is washed by injecting 0.5 kg of palm olein. The cake is then pressed up to a pressure of 15 bar. 2 kg of a slightly cloudy oil are thus recovered, which oil is filtered on the filter
25 press with a 1 micron filter. The P content of the filtered oil, which is up to 66 mg/kg, is determined. The filtered oil is subjected to physical refining. With the aim of reducing the phosphorus content, the oil is stirred at 85°C for 20 min with 2% Trisyl®, and
30 then dried at 85°C under a 20 mbar vacuum and filtered. Finally, the oil is deodorized at 180°C/1 mbar for 3 hours. The refined oil is analysed in order to determine its purity (FFA, P) and the level of incorporation of the DHA. The results are summarized in
35 Table 3 below.

Table 3

% DHA	4.9
Level of incorporation of DHA (%)	96.5
% FFA	0.07
mg/kg P	2

5 Example 4: Physical refining of the mixture of pressed
oils containing DHA

Equipment:

- 1 l laboratory glass reactor
Seitz 2 l filter press
10 Laboratory deodorizer

Materials used:

Trisyl® (processing agent)

15 Procedure:

With the aim of obtaining a refined oil with a DHA concentration of about 8%, 50 g of pressed oil as obtained in Example 2 and 500 g of pressed oil as obtained in Example 3 before the refining step are
20 mixed in the reactor. The mixture is stirred at 85°C for 20 min with 2% of Trisyl®, and then dried at 85°C under a 20 mbar vacuum, and finally filtered. 540 g of a clear oil are thus recovered.

300 g of this oil are introduced into the deodorizer
25 and treated with steam for 3 hours at 180°C/1 mbar. The refined oil is analysed in order to determine its purity (FFA, P) and the DHA content. The results are summarized in Table 4 below.

Table 4

30

% DHA	8
% FFA	0.07
mg/kg P	2

Example 5: Incorporation into palm olein of DHA and ARA obtained from biomasses

5 Equipment:

Fryma MZ 80 colloid mill

De Smet minipilot filtration unit with 1.4 l chamber

Seitz 2 l filter press

Laboratory deodorizer

10

Materials used:

Biomass containing 36% of oil containing 43.1% of arachidonic acid (ARA)

15 Biomass containing 50.5% of oil containing 39% of decosaheanoic acid (DHA)

Palm olein

Trisyl® (processing agent)

Procedure:

20 0.7 kg of the biomass containing DHA, 0.7 kg of the biomass containing ARA and 2 kg of palm olein are introduced into the container of the mill. The milling is carried out by direct passage through the mill with a slit of 10 microns at a temperature of 40-50°C and

25 the mixture is recovered. 2.5 kg of this mixture are introduced into the filtration unit and filtration is carried out at 50°C. The cake is washed by injecting 0.92kg of palm olein. The cake is then pressed up to a pressure of 15 bar. 2.5 kg of a slightly cloudy oil are

30 thus recovered, which oil is filtered on the filter press with a 1 micron filter. The P content of the filtered oil, which is up to 26 mg/kg, is determined. The filtered oil is subjected to physical refining. With the aim of reducing the phosphorus content, the

35 oil is stirred at 85°C for 20 min with 1% Trisyl®, dried at 85°C under a 20 mbar vacuum and then filtered. Finally, the oil is deodorized at 180°C/1 mbar for 3 hours. The refined oil is analysed in order to determine its purity (FFA, P) and the level of

incorporation of the DHA and of the ARA. The results are summarized in Table 5 below.

Table 5

5

% DHA	2.3
Level of incorporation of DHA (%)	64.6
% ARA	2.8
Level of incorporation of ARA (%)	99
% FFA	0.04
mg/kg P	2

Examples 6-8

6. An infant formula for premature babies enriched with ARA is prepared from oil prepared by the process of Example 1 and a fish oil enriched with DHA, containing about 24% of DHA and there are added thereto other oils, for example in the proportions indicated in Table 6 below, proteins, where appropriate hydrolysed, carbohydrates, and where appropriate vitamins and trace elements.

Table 6

	Example 6
Oil from Example 1	0.8
Fish oil	1.3
MCT oil	27
Soyabean oil	23
Palm olein	47.9
Total	100

20

7. An infant formula for full-term unweaned babies enriched with ARA and with DHA is prepared from the carrier oil prepared by the process of Example 5 and

there are added thereto other oils, for example in the proportions indicated in Table 7 below, proteins, where appropriate hydrolysed, carbohydrates and where appropriate vitamins and trace elements.

5

Table 7

	Example 7
Oil from Example 5	13
Coconut oil	20
Soyabean oil	20
Palm olein	47
Total	100

8. A follow-on milk for small children enriched with DHA is prepared from the carrier oil prepared by the process of Example 4, and there are added thereto other oils in the proportions indicated in Table 8 below, proteins, where appropriate hydrolysed, carbohydrates and where appropriate vitamins and trace elements.

15

Table 8

	Example 8
Oil from Example 4	4
Palm kernel oil	27
Soyabean oil	23
Palm olein	46
Total	100

Example 9

20

A liquid milk enriched with DHA in an amount of 1% of DHA in the fatty phase is prepared in the following manner:

A whole milk containing 3.92% of fat and 8.58% of solids-not-fat and a low-fat milk containing 0.05% of fat and 9% of solids-not-fat are pasteurized separately

25

by treating them at 87°C for 12 s.

34.69 kg of whole milk and 160.26 kg of low-fat milk, cooled to 15°C, are then mixed, and then a premix of
5 0.77 kg of oil obtained according to Example 3 (palm olein, containing 4.9% of DHA), 1.6 kg of soyabean oil and 1 g of vitamin E heated to 50°C is incorporated into this mixture by means of a colloid mill..

10 Sterilized product:

After heating to 80°C in a plate exchanger, the liquid is UHT sterilized at 148°C for 5 s. After cooling at 78°C, it is homogenized in two stages, at 200 bar, and then at 50 bar; it is cooled to 20°C and it is
15 aseptically packaged in carton-type packaging which has been previously sterilized, the homogenization, cooling and filling stages taking place aseptically.

Pasteurized product:

20 The liquid is heated at 72°C for 15 s in a plate exchanger; it is homogenized in two stages at 200 bar, and then at 50 bar; it is cooled to 4°C and it is packaged in carton-type packaging.

25 Example 10

As a nutritional supplement, an oil prepared according to Example 1 containing ARA or an oil prepared according to Example 2 containing DHA is encapsulated
30 in an amount of 500 mg of oil in gelatin capsules.

Claims

1. Process for preparing a stable oil containing long-chain polyunsaturated fatty acids (LC-PUFAs) in the form of triacylglycerols, in particular arachidonic acid (ARA), dihomogammalinolenic acid (DHGLA), docosaheptaenoic acid (DHA) or eicosapentaenoic acid (EPA), characterized in that one or more biomasses obtained from the culture of a microorganism, especially of a fungus or of a microalga containing the acids ARA, DHGLA, DHA or EPA, are pressed in the dry state so as to obtain a first press oil and a cake, and in that the oil thus obtained is treated with an adsorbent and in that it is subjected to deodorization under controlled conditions.
2. Process according to Claim 1, in which the biomass contains at least one long-chain polyunsaturated fatty acid chosen from ARA and DHA.
3. Process according to Claim 2, in which a biomass containing ARA is treated.
4. Process according to Claim 2, in which a biomass containing DHA is treated.
5. Process according to Claim 2, in which a mixture of biomasses, containing ARA and DHA, is treated.
6. Process according to one of Claims 1 to 5, in which a carrier oil, entering into the composition of a food, nutritional, pharmaceutical or cosmetic product, is brought into contact with the press cake of the biomass so as to transfer the long-chain polyunsaturated fatty acid(s) in the form of triacylglycerols to the said carrier, the oil containing the said fatty acid(s) is separated from the biomass cake by pressing and filtration, which constitutes the second press oil, and the pressed oils

are combined and they are refined under controlled conditions.

- 5 7. Process according to Claim 6, in which the pressed oils are subjected to physical refining using a processing agent, it being possible for the treatment to be carried out during contact with the carrier oil or after production of the pressed oil, in particular during filtration.
- 10 8. Process according to Claim 1, in which the walls of the cells of the microorganisms are broken by pressing, in order to increase the level of incorporation of the biomass oil into the carrier oil.
- 15 9. Process according to Claim 6, in which the press cake of the biomass is subjected to grinding in the presence of the carrier oil under gentle conditions, at a moderate temperature under an inert atmosphere, in particular under a nitrogen layer.
- 20 10. Process according to one of Claims 6 to 9, in which a final filtration is carried out in order to remove the fine particles of biomass residue.
- 25 11. Foodstuff containing an oil obtained by the process according to one of Claims 1 to 10.
- 30 12. Infant foodstuff containing an oil obtained by the process according to one of Claims 1 to 10.
- 35 13. Infant foodstuff containing an oil obtained by the process according to one of Claims 1 to 10, in combination with a fish oil.
14. Nutritional composition containing an oil obtained by the process according to one of Claims 1 to 10.
15. Cosmetic composition in dry or emulsion form

containing an oil obtained by the process according to one of Claims 1 to 10.

16. Foodstuff containing an oil obtained according to
5 one of Claims 1 to 10 intended as animal feed.

17. Foodstuff containing the biomass residue obtained
by the process according to one of Claims 1 to 10,
intended as animal feed.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 03/08745

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C11B1/10 C11B1/06 A23D9/00 C12P7/64 A23L1/30
A23K1/16 A23C11/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C11B A23D C12P A23L A23K A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	EP 1 239 022 A (NESTLE SA) 11 September 2002 (2002-09-11) the whole document	1-17
X	EP 1 178 118 A (DSM NV) 6 February 2002 (2002-02-06) paragraphs '0002!', '0005!', '0006!', '0008! - '0014!', '0016!', '0024!', '0026!', '0027!; claims 1-7	1-17
X	WO 97/37032 A (WOLF JOHANNES HENDRIK ;SCHAAP ALBERT (NL); BIJL HENDRIK LOUIS (NL)) 9 October 1997 (1997-10-09) cited in the application	11-17
A	page 13, line 19 - page 14, line 27; claims; examples 16,17,19	1-10
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *8* document member of the same patent family

Date of the actual completion of the international search

17 December 2003

Date of mailing of the international search report

29/12/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Vernier, F

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/08745

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE WPI Section Ch, Week 198426 Derwent Publications Ltd., London, GB; Class C03, AN 1984-164291 XP002175534 & SU 1 049 431 A (PLANT PHYSIOL INST) 23 October 1983 (1983-10-23) abstract</p>	17
A	<p>WO 92/12086 A (GERBER GARMENT TECHNOLOGY INC) 23 July 1992 (1992-07-23) the whole document</p>	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/08745

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 1239022	A	11-09-2002	EP 1239022 A1	11-09-2002
			CA 2438601 A1	19-09-2002
			WO 02072742 A1	19-09-2002
			NO 20033894 A	03-09-2003
EP 1178118	A	06-02-2002	EP 1178118 A1	06-02-2002
			AU 9371101 A	13-02-2002
			BR 0112942 A	08-07-2003
			CA 2417571 A1	07-02-2002
			CN 1447860 T	08-10-2003
			WO 0210423 A2	07-02-2002
			EP 1305440 A2	02-05-2003
			NO 20030510 A	31-03-2003
WO 9737032	A	09-10-1997	AU 731785 B2	05-04-2001
			AU 2159297 A	22-10-1997
			CA 2250581 A1	09-10-1997
			CN 1217029 A	19-05-1999
			WO 9737032 A2	09-10-1997
			EP 0894142 A2	03-02-1999
			ID 16504 A	02-10-1997
			JP 2000508888 T	18-07-2000
			US 2001025114 A1	27-09-2001
			US 6255505 B1	03-07-2001
			ZA 9702702 A	27-03-1998
			AT 249514 T	15-09-2003
			AU 733734 B2	24-05-2001
			AU 2505097 A	22-10-1997
			CA 2250575 A1	09-10-1997
			CN 1226923 A	25-08-1999
			DE 69724782 D1	16-10-2003
			WO 9736996 A2	09-10-1997
			EP 1369474 A1	10-12-2003
			EP 0906414 A2	07-04-1999
			ID 16503 A	02-10-1997
			JP 2002502233 T	22-01-2002
			US 2003143659 A1	31-07-2003
			ZA 9702706 A	22-10-1997
SU 1049431	A	23-10-1983	SU 1049431 A1	23-10-1983
WO 9212086	A	23-07-1992	EP 0566649 A1	27-10-1993
			JP 2525109 B2	14-08-1996
			JP 5509066 T	16-12-1993
			WO 9212086 A1	23-07-1992
			US 5256238 A	26-10-1993